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ScienceDirect
Fungal Genetics and Biology 44 (2007) 323–329
FUNGAL GENETICS AND BIOLOGY
www.elsevier.com/locate/yfgbi

Genetic mapping of *Pyrenophora teres* f. *teres* genes conferring avirulence on barley

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Received 18 May 2006; accepted 27 November 2006
Available online 16 January 2007

Abstract

A *Pyrenophora teres* f. *teres* cross between isolates 0–1 and 15A was used to evaluate the genetics of avirulence associated with barley lines Canadian Lake Shore (CLS), Tifang, and Prato. 15A is avirulent on Tifang and CLS, but virulent on Prato. Conversely, 0–1 is avirulent on Prato, but virulent on Tifang and CLS. Avirulence:virulence on Tifang and CLS segregated 1:1, whereas avirulence:virulence on Prato segregated 3:1. An AFLP-based linkage map was constructed and used to identify a single locus derived from 15A (*AvrHar*) conferring avirulence to Tifang and CLS. Virulence on Prato was conferred by two epistatic genes (*AvrPra1* and *AvrPra2*). *AvrPra2* co-segregated with *AvrHar*, but the two genes from opposite parents conferred opposite reactions. This work provides the foundation for the isolation of these avirulence genes.
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Keywords: Barley; Net blotch; Quantitative trait loci; Avirulence; AFLP

1. Introduction

Barley net blotch caused by the Ascomycete fungus *Pyrenophora teres* f. *teres* Smedeg. [Anamorph: *Drechslera teres* (Sacc.) Shoem. f. *teres* Smedge.], is one of the most widely distributed foliar diseases of barley (*Hordeum vulgare* L.). It is most severe in temperate regions of high rainfall and humidity, but epidemics have occurred in low rainfall areas as well (Steffenson and Webster, 1992). The pathogen causes lesions that initially appear as spots and short yellow streaks on leaves, and the lesions can expand into longer longitudinal and transverse necrotic streaks on susceptible genotypes. Typical yield losses due to net blotch outbreaks range from 10% to 40% (Mathre, 1997). *Pyrenophora teres* f. *teres* is a heterothallic haploid fungus that produces pseudothecia which contain ascospores. Ascospores are forcibly discharged early in the growing season and are generally thought to be the primary inoculum for net blotch disease induction. During most of its life cycle, *P. teres* f. *teres* produces haploid asexual conidia that are the source of secondary inoculum. Conidia are dispersed by wind and rain to surrounding leaves producing disease throughout the growing season when environmental conditions are favorable.

According to the gene-for-gene model (Flor, 1956), disease responses in many plant-pathogen interactions result from the expression of a resistance (*R*) gene in the plant and a corresponding avirulence (*Avr*) gene in the pathogen. When a pathogen carrying an *Avr* gene tries to infect the host carrying the corresponding *R* gene, the *Avr* gene product (Avr) can act as an elicitor, or it can direct the synthesis or modify a metabolite or protein that is a race-specific elicitor. The elicitor can be recognized by a receptor (putative product of the matching *R* gene) of the resistant plant. This interaction would activate a signal transduction pathway and finally lead to

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1087-1845/$ - see front matter Published by Elsevier Inc.
doi:10.1016/j.fgb.2006.11.009

active resistance (Leach and White, 1996; Laugé et al., 1998). Although fungal diseases are a major challenge for important crops, few avirulence genes have been characterized and their structures are highly diverse (Böhnert et al., 2004). The first two cloned fungal avirulence genes: *AVR9* and *AVR4* were isolated from *Cladosporium fulvum* (van Kan et al., 1991; Joosten et al., 1994). *AVR9* and *AVR4* are secreted by the fungus during the infection process and efficiently trigger resistance responses in the cultivars carrying the corresponding *R* gene: *cf-4* and *cf-9*, respectively. Another avirulence gene (*NIP1*) was cloned from the barley pathogen *Rhynchosporium secalis*. The product of *NIP1* is a phytotoxic peptide that acts as an elicitor of the defense response in barley cultivars carrying the resistance gene, *Rrs1* (Rohe et al., 1995) and is responsible for the induction of necrosis. In *Magnaporthe grisea*, four avirulence genes (*AVR-pita*, *PWL2*, *ACE1* and *AVR1-CO39*) have been cloned (Yasuda et al., 2005). The AVR-Pita protein produces a hypersensitive response in rice plants with the *Pita* gene but not in plants without the *Pita* gene. *Avr-Pita* functions as an elicitor molecule that directly binds the Pita protein and triggers a signal transduction cascade leading to resistance (Jia et al., 2000). *ACE1* (Avirulence Conferring Enzyme1) encoding a putative hybrid between a polyketide synthase, and a nonribosomal peptide synthetase enzyme expresses exclusively during the fungal penetration of leaves. Isolates carrying *ACE1* are specifically recognized by the rice cultivars carrying the corresponding resistance gene *Pi33* (Böhnert et al., 2004). Four avirulence genes have been identified from *Melampsora lini* (flax rust). These include *AvrL567*, *AvrM*, *AvrP4* and *AvrP123* (Dodds et al., 2004; Catanzariti et al., 2006). Transient expression of these genes in flax caused resistance gene-mediated cell death (Catanzariti et al., 2006). Further study on *AvrL567* discovered that *AvrL567* sequence divergence lead to qualitative differences in recognition specificity by the corresponding *R* genes (Dodds et al., 2006). Two avirulence genes, *AVRa10* and *AVRk1*, have been identified from *Blumeria graminis* f sp *hordei* causal agent of barley powdery mildew. These genes belong to a large family with more than 30 paralogues indicating that a repertoire of *Avr* genes existing in the *B. graminis* population enable this pathogen to overcome host *R* genes rapidly (Ridout et al., 2006). Nine avirulence genes (*AvrLm1-9*) have been mapped in *Leptosphaeria maculans*, causal agent of stem canker on oilseed rape, with *AvrLm1* being cloned and characterized (Gout et al., 2006).

Barley resistance to *P. teres* f. *teres* has been reported in both quantitative (Arabi et al., 1990; Steffenson and Webster, 1992; Robinson and Jalli, 1997; Robinson, 1999) and qualitative forms (Schaller, 1955; Mode and Schaller, 1958; Kahn and Boyd, 1969; Bockelman et al., 1977; Wilcoxson et al., 1992; Steffenson et al., 1996; Manninen et al., 2000; Cakir et al., 2003; Friesen et al., 2006). The presence of qualitative resistance suggests the potential for a gene for gene interaction occurring in this pathosystem. Although *P. teres* f. *teres* causes an economically devastating disease and a fair amount of research has been done on host resistance, few studies specifically investigating the genetics conditioning pathogenicity have been conducted.

The avirulence gene *AvrHar*, specific to barley cultivar 'Harbin', was linked to five random amplified polymorphic DNA (RAPD) markers (Weiland et al., 1999). *AvrHar* conferred *P. teres* f. *teres* avirulence to Harbin barley. This result was consistent with previous reports that Harbin carried dominant genes for resistance to *P. teres* f. *teres* (Mode and Schaller, 1958). According to the gene-for-gene hypothesis, *AvrHar* probably corresponds to a dominant resistance gene in Harbin barley.

Several pathotype studies have focused on the variability of virulence in *P. teres* in field populations (Jalli, 2004; Wu et al., 2003; Cromey and Parks, 2003; Gupta and Loughman, 2001; Jonsson et al., 1997; Steffenson and Webster, 1992; Tekauz, 1990; Kahn, 1982; Kahn and Boyd, 1969). Based on the resistance genes present in the differential sets used, a high level of variability was identified in worldwide collections of *P. teres* indicating that many different virulence factors are present in the population. These factors could be either virulence genes used to induce infection, avirulence genes recognized by the host to induce resistance or a combination of these mechanisms. Based on the common differential lines used in these previous studies it is estimated that as many as 20 resistance genes could be present in cultivated barley germplasm. There is much to be learned about the genetics of pathogenicity and the cause of the various observed phenotypes in *P. teres*, as few such studies have been performed.

Although it is labor intensive, up to now, map-based cloning is still the primary method used to clone genes of interest and requires the generation of a saturated linkage map. Many different kinds of molecular techniques including restriction fragment length polymorphism (RFLP) (Botstein et al., 1980), random amplified polymorphic DNA (RAPD) (Williams et al., 1990) and amplified fragment length polymorphism (AFLP) (Vos et al., 1995) have been used to construct genetic linkage maps in fungi. Because of its simplicity, stability and polymorphism detection frequency, AFLP has been a popular tool for constructing recombination based genetic linkage maps in fungi such as *Leptosphaeria maculans*, *Gibberella zeae*, and *Cochliobolus sativus* (Attard et al., 2002; Jurgenson et al., 2002; Cumagun et al., 2004; Zhong et al., 2002).

In this study, a fungal population generated from a cross of *P. teres* f *teres* isolates that showed differential disease reactions on multiple barley genotypes was used for the genetic characterization of avirulence genes in *P. teres* f. *teres*. An AFLP-based genetic linkage map of the *P. teres* f. *teres* population was developed and AFLP markers were used to establish the relationships between the disease phenotypes and the genetic map to identify genes associated with virulence/avirulence in *P. teres* f. *teres*. The close linkage of molecular markers to these genes will provide a